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(54) Title: PORCINE POLYCLONAL AND MONOCLONAL ANTIBODIES

(57) Abstract

This invention pertains to porcine polyclonal and monoclonal antibodies reactive with a predetermined antigen and methods of preparing the same. The porcine antibodies are useful in therapeutic methods for treating antigen mediated diseases.

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PORCINE POLYCLONAL AND MONOCLONAL ANTIBODIES

Background of the Invention

Murine monoclonal antibodies have been used for the diagnosis and treatment of a multitude of human diseases. One murine monoclonal antibody, OKT 3, has been described as a potent immunosuppressive agent in organ allotransplantation during rejection episodes (Ortho Multicenter Transplant Study Group; New Eng. J. Med. 313:337, 1985), and as useful in preventing the onset of rejection (Kreis, H. et al., Transplant Proc. 17:2734, 1985). OKT 3 binds to mature human T cells, binding tightly to the T-cell receptor (Moner, S.C. et al., J. Exp. Med. 157:705, 1983).

Despite some initial optimism, the injection of murine proteins into a human can provoke immune response by the host. Allergic reactions provoked can include fever, rash, anaphylaxis, and serum sickness. This phenomenon has acquired increased importance concurrent with the intensified efforts to utilize murine monoclonal antibodies in clinical medicine. (Cosimi, A.B., et al., N. Eng. J. Med. 305:308, 1981; Miller, R.A., et al., Blood 58:78, 1981; Sears, H.G., et al., Lancet 2:762, 1982; Dillman, R.O., et al., Blood 59:1036, 1982; Colvin, R.B., et al., Fed. Proc. 41:363, 1982; Jaffers, G.J.,

et al., Transplant Proc. 15:643, 1983; Koprowski H., et al., Proc. Natl. Acad. Sci. USA 81:216, 1984; Baudrihaye, M.F., et al., Europ. J. Immunol. 8:686, 1984; and Thistlethwaite, J.R., et al., Transplantation 38:695, 1984).

The initial effect of the administration of murine monoclonal antibodies in humans, e.g., as antitumor agents, is often dramatic; however, the effect can wane because of the host's escape mechanism such as, antigenic modulation, and antimonoclonal xenosensitization (Nadler, L.M., et al., Cancer Res. 40:3147, 1980; Ritz, F., et al., Blood 58:141, 1981; Miller, R.A., et al., Lancet 2:226, 1981; Miller, R.A., et al., N. Eng. J. Med. 306:517, 1982; Levy, R., et al., Fed. Am. Soc. Exp. Biol. 42:2650, 1983; and Miller, R.A., et al., Blood 62:988, 1983). Murine/human hybridomas and human/human hybridomas have been experimented with in an effort to eliminate the xenogeneic effect with limited success. Moreover, for obvious reasons, one can only use human/human hybridomas in a very limited number of cases.

Summary of the Invention

This invention pertains to the production of porcine antibodies reactive with a predetermined antigen, preferably porcine monoclonal antibodies. The porcine antibodies of this invention are produced by immunizing a pig with an antigen under conditions whereby porcine antibodies are produced. When porcine polyclonal antibodies are desired, blood is collected from the pig and the serum containing the

polyclonal antibodies is separated from the other blood components.

Porcine monoclonal antibodies are produced by recovering porcine antibody-producing cells from the pig's spleen and fusing them with immortalizing cells thereby producing hybridomas thereof. The hybridomas capable of producing monoclonal antibodies reactive with the predetermined antigen are cultured and cloned and the porcine monoclonal antibodies are recovered from the hybridoma supernate.

The porcine antibodies of this invention have an advantage over presently used murine antibodies in that the porcine antibodies have less of an immunogenic effect when administered to a human.

Detailed Description

The genetic resemblance between porcine and human proteins is well known in regards to insulin. Furthermore, porcine immunoglobulins bear striking resemblance to human immunoglobulins. This invention pertains to the discovery that the pig is a useful source of antibody-producing cells used in the production of various porcine/porcine hybridomas and porcine/human hybridomas. Hybridomas with other species can also be prepared, (e.g., simian/human hybridomas). However, the limited access to primates such as humans and monkeys for immunization with various antigens prevents general use of these animals for the preparation of hybridomas and monoclonal antibodies. Employing pigs as the source of antibody-producing cells can decrease the risk of xenosensitization.

The term "monoclonal antibody" encompasses whole antibodies or biologically functional fragments

thereof. Fragments typically include that portion of the antibody capable of binding to the respective antigen.

Monoclonal antibody also includes chimeric antibodies comprising portions derived from two different species (e.g., human constant region and porcine binding portion). The portions derived from two different species can be joined together chemically by conventional techniques, can be prepared as a single protein using genetic engineering techniques, can be produced by genetic rearrangements or can be produced by other techniques. DNA encoding the proteins of both portions of the chimeric antibody can be inserted into a vector and the chimeric antibody can be expressed as a continguous protein.

A strain or breed of pig capable of producing immunoglobins having less of an immunogenic effect than murine immunoglobins when administered to a human can be used in this invention. The preferred pig being the mixed Yorkshire breed pigs. Examples of other breeds of pigs which can be used include, the Duroc, Hampshire, Spotted Swine, Poland China, Chester White, Berkshire, O.I.C., Hereford, Tamworth, or minipig breeds.

Antigens which can be used for immunization of the pig include retroviruses such as HIV-1, HIV-2, or HTLV-1, cytomegalovirus (CMV), a hepatitis virus such as Hepatitis B virus, a rabies virus, or other types of viruses including various oncogene viruses; Tumor Necrosis Factor-alpha (TNF-alpha); antigens from pathogenic bacteria, e.g., bacteria such as E. coli, which produce sepsis, or other microorganisms. Various tumor antigens can also be used as immunogens to obtain monoclonal antibodies for use in anti-tumor

treatment. Alternatively, T3 lymphocytes can be used as immunogens, for the purpose of preparing T3 lymphocyte monoclonal antibodies useful for treating or preventing organ rejection after transplants.

This invention encompasses a method for the preparation of antibodies reactive with a predetermined antigen, comprising:

a) immunizing a pig with the antigen under conditions whereby antibodies reactive with the antigen are produced;

b) harvesting cells containing the antibodies reactive with the antigen by collecting blood from the pig; and

c) separating the serum containing the antibodies from the other blood components.

Upon recovery and purification, the antibodies can be directly employed in polyclonal form by administering a therapeutically effective dosage to a patient as part of a passive immunotherapeutic method of treating a disease associated with the antigen. A therapeutically effective dosage is that dosage which significantly reduces or eliminates symptoms of the disease being treated.

Alternatively, the antibodies can be administered in the form of monoclonal antibodies. Monoclonal antibodies can be produced using somatic cell hybridization techniques (Kohler and Milstein, Nature (1975)). Hybridomas capable of producing monoclonal antibodies reactive with the predetermined antigen are produced by:

a) immunizing a pig with the antigen under conditions whereby antibodies reactive with the antigen are produced;

b) recovering the antibody producing cells from the pig's spleen;

d) fusing the porcine antibody producing cells with immortalizing cells (porcine, human, or other primate);

e) eliminating the unfused, residual porcine antibody producing cells and immortalizing cells; and

f) selecting the desired hybridomas capable of producing monoclonal antibodies reactive with the desired antigen.

In accordance with a further feature of the invention, anti-idiotype antibodies to the predetermined antigen are prepared by;

a) immunizing a first breed of animal, e.g., a first breed of pig, with the antigen under conditions whereby antibodies reactive with the antigen are produced;

b) harvesting the antibodies reactive with the antigen by collecting blood from the first animal when the antibodies reach a desired titer;

c) separating the antibodies from the blood;

d) immunizing a second breed of animal, e.g., a second pig breed, with the antibodies; and

e) recovering the resulting anti-antibodies for use as a vaccine.

As indicated more fully below, the use of anti-idiotype antibodies reduces xenosensitization, and, therefore, can be useful for human therapy.

In accordance with yet a further feature of the invention, the risks implicit in the use of murine

monoclonal antibodies in human therapy can be decreased, and possibly eliminated, by translating murine monoclonal antibodies to "porcine/porcine" or "porcine/human" monoclonals, utilizing the techniques described above and employing the murine monoclonal antibodies to initially immunize the pig. Alternatively, porcine/porcine or porcine/human monoclonal antibodies prepared as described hereinabove are administered by a "sandwich technique" concurrent with murine monoclonal antibody therapy, to diminish or possibly eliminate xenosensitization produced by the latter.

These and other features and advantages of the present invention will be more fully apparent from the following detailed description of preferred embodiments thereof.

Preparation of Porcine-Man Hybridomas

To prepare the monoclonal antibodies of the invention, a pig is immunized with the desired antigen or antigens, and boostered until a sufficient titer is obtained of the antibody(s) reactive with the antigen(s) with which the pig was immunized. Sufficiency of the titer of the antibodies obtained can be determined using conventional techniques. For example, when anti-gp 48 antibodies to HIV-1 are produced (see parent application Serial No. 215,867), a titer at least equal to that of a low positive reference (e.g., a standard HIV-1 positive control) is sufficient. When measured by use of an Electronucleonics ELISA test kit with a Behring ELISA Processor II (at 492 nm), such a low positive reference value is about 0.7. Suitable titers of other

antibodies can be determined employing other commercially available assays, e.g., ELISA CMV or Hepatitis B antibody test kits for CMV and Hepatitis B antibodies, respectively.

When an adequate titer is thus determined, the immunocytes can be recovered. Plasma cells and other pertinent immunocytes can be obtained from blood or other organs such as the bone marrow. The spleen is the preferred organ to use for harvesting the immunocytes.

The spleen can be recovered when IgM type specific antibodies peak, i.e., about 8-20 days, preferably 12-17 days, post-vaccination, or after one or several booster doses have been administered to the pig. When IgG antibodies are preferred, the pig is given about 3 to 5 vaccinations spaced about 14 to 30 days apart, the maximum antibody titer being obtained about 2 months after the initial vaccination. Preferably, the pig is thereafter given a split booster dose, both subcutaneously (s.c.) and intraperitoneally (i.p.), 2-5 days prior to sacrificing the pig. The spleen is thereafter harvested and the immunocytes are recovered.

The porcine immunocytes are then fused with immortalizing cells (e.g., myeloma cells) to form hybrid cells (herein after hybridoma). An immortalizing cell is a cell capable of living for long periods of time in cell culture media. Porcine/porcine (malignant plasma cells), or porcine/primate hybrids such as porcine/simian hybridomas are preferred. The porcine immunocytes are most preferably fused with human myeloma cells or like sub-types. GM4672B human myeloma cells (EMC modified CM1500

myeloma cells) have been found to be particularly suitable. This cell line is an IgG2 kappa secreting cell line which previously has been used for human/human hybridoma production. Alternatively, other myeloma cell lines, e.g., IgM secreting or other IgG secreting cell lines, can be employed. Advantageously, myeloma cells which are sensitive to medium (HAT) hypoxanthine: aminopterin: thymidine, such as the G4672B cell line, can be used.

To form the hybridoma, the spleen from the immunized pig is minced, and the plasma cells (immunocytes) are washed in media such as RPMI 1640 or IMDM and fused with the appropriate human myeloma cells, using polyethylene glycol (PEG) to effect fusion. For fusion, a modification of the protocols described by Oi, et al., and Foster (Oi, V.T., Herzenberg, L.A., In B.B., Mishell and S.M., Shiigi (eds), Selected Methods in Cellular Immunology, pp. 351-372, San Francisco, W.H. Freeman Co. 1980; Foster, C., Cancer Treat. Rev. 9:59, 1982) can be utilized. The PEG used for fusion can be 30-50% PEG 1500, and the cells can be incubated with the PEG for varying periods.

Desirable fusion frequency can be obtained at a PEG concentration of about 30-35%, and at temperatures varying from about ambient room temperature to 40°C. When using room temperature, PEG incubation times can be about 7-9 minutes. If PEG is used at 40°C, a shorter incubation time can be advantageous. For example, use of the protocol of Galfre and Milstein (1981), results in reasonable high fusion frequencies.

After fusion the cells are distributed, e.g., in 24 well plates, and HAT medium is added every two to three days with replacement of 50% of the supernatant fluid concurrent with each HAT addition. The human myeloma cells are eliminated by the HAT medium, and the porcine plasma cells not yet hybridized die spontaneously in a few days.

The appearance of hybridoma clones is observed microscopically until significant hybridoma clones are observed. The hybridomas are explanted to 24 well plates and allowed to grow to a density of about 10^5 cells/well. The supernate is tested for the presence of antibodies reactive with the antigen(s) used for immunization of the pig. In the same manner as indicated hereinabove, e.g., CMV or Hepatitis B antibodies are readily determined by testing on commercially available ELISA CMV or Hepatitis B antibody test kits. Test kits for the determination of specific human antibodies can be used since both porcine immunoglobulin and human immunoglobulin - as well as porcine/human antibodies - can be detected using antihuman antibodies. The type of immunoglobulin IgM or IgG can be detected using antihuman (or antiporcine) immunoglobulin tests such as Nor Partigen plates (Behring Diagnostic). Cloning by limiting dilution is then performed (Oi _et al._, _cited supra_, 1980).

Production of Porcine Anti-idotype Antibodies Reactive with Murine Monoclonal Antibodies

The pig can be immunized with a known murine monoclonal antibody, e.g., of any of the IgM, IgG1, IgG2a, or IgG2b types, to produce an anti-antibody in

the pig reactive with the respective type of immunoglobulin. The antibodies in the porcine blood can be utilized as a polyclonal antibody against the different classes of murine immunoglobulin. Further, hybridomas can be produced by hybridizing an immunocyte from the pig with an immortalizing cell. The hybridomas (porcine/porcine, porcine/human) produce monoclonal antibodies reactive with the monoclonal used as an immunogen. The monoclonal antibodies can be utilized in diagnostic assays or in therapy.

Murine monoclonal antibodies which are specific, e.g., to certain tumor antigens, are administered intravenously and bind to the tumor antigens toward which the particular murine monoclonal is "targeted". Binding to the target antigens occurs relatively rapidly after injection of the murine monoclonal antibody. Shortly thereafter, the porcine antimurine antibody, either a polyclonal antibody or a monoclonal antibody, is administered intravenously. The porcine anti-murine immunoglobulin or porcine monoclonal anti murine antibody then targets the previously administered murine monoclonal antitumor antibody and binds to the murine monoclonal. The porcine antibody can be flagged by a radioisotope and the location of the tumor(s) to which the murine monoclonal has been bound can then identified upon scanning the patient.

This sandwich technique has two advantages over administration of a murine monoclonal antibody alone. First, the "sandwich" bound antibody provides a stronger signal than the murine monoclonal. Second, and even more important, use of the sandwich

technique imposes a much lower risk that the patient may produce an anti-murine immunoglobulin antibody, the porcine/human hybridoma in particular binding to the "antigenic determinants" to the murine antibody and thus reducing significantly or possibly eliminating xenosensitization.

In attempts to treat patients with murine monoclonal antibodies, be it treatment of transplant patients to prevent organ rejection (Bach, J.F., et al., Transplantation Proceedings 19: (Suppl.1) pp. 17-20, 1987), or in other diseases in which murine monoclonal antibodies are used to provoke an immune response by the host (Cosimi, A.B., et al., N. Eng. J. Med., 305:308, 1981; Cosimi, A.B., et al., Transplantation, 32:535, 1981; Miller, R.A., et al., Blood 58:78, 1981; Sears, H.G., et al., Lancet 2:762, 1982; Dillman, R.D., et al., Blood 59:1036, 1981; Colvin, R.B., et al., Fed. Proc. 41:363, 1982; Jaffers, G.J., Transpl. Proc. 15:646, 1983; Chatenoud, L., et al., Transpl. Proc. 15:643, 1983; Koprowski, H., et al., Proc. Natl. Acad. Sci., USA, 81:216, 1984; Baudrihaye, M.F., et al., Eur. J. Immunol 8:686, 1984; and Thistlethwaite, J.R., et al., Transplantation 38:695, 1984) xenosensitization of the host can be prevented by "neutralization" of antigenic determinants on the murine monoclonal antibodies. The method of the present invention makes it possible to use murine monoclonal antibodies in humans for longer periods without production of host anti-murine antibodies which otherwise prevent further use of the monoclonal antibodies.

When the pig is immunized with the murine immunogloblin type which is to be used in humans, it

can either be sacrificed after one vaccination plus a booster dose (if IgM antibodies are preferred), or after repeated vaccinations (if IgG antibodies are desired), as described more fully hereinabove. After purifying the porcine immunoglobulins, the porcine serum can be used as a polyclonal IgM or IgG.

The polyclonal antibodies can be further purified by passing the porcine serum or partially purified immunoglobulin through a Sepharose CNBR-4B affinity column to which the murine monoclonal antibody is coupled. The porcine antibody binds to the murine monoclonal antibody in the column. The purified porcine anti-murine monoclonal antibody or polyclonal antibody can then be eluted, the resulting antibody having high specificity to a murine monoclonal antibody of the type against which the pig has been immunized. The porcine polyclonal antibody can be used _in vivo_ to bind and neutralize the murine antibody's antigenic determinants and thus prevent xenoxensitization.

Although the porcine immunoglobulins are better accepted than murine immunoglobulins by the human host's immunosurveillance, there is of course a possibility of xenosensitization against the porcine immunoglobulins. To prevent any such xenosensitization it is advantageous to harvest the porcine immunocytes from the immunized pig and produce a porcine/human hybridoma or a porcine/simian hybridoma to obtain a monoclonal antibody against the murine monoclonal antibody, and utilize the resulting monoclonal antibody in combination with the murine monoclonal.

When the murine monoclonal antibodies are prepared as anti-idiotype antibodies for production of various prophylactic vaccines (Gaulton, G.N., et al., J. Immunology 137:2930, 1986) or in treatment of autoimmune diseases (Agius, M., Richman, D.P., J. Immunology 137:2195, 1986), the porcine antibodies can be used to suppress xenosensitization in the host.

It is thus within the scope of this invention to use the pig to produce antibodies against any type of monoclonal antibody from those species of animals from which monoclonal antibodies have been produced, e.g., against rat monoclonal antibodies, or against any such types of polyclonal antibodies, e.g., against horse anti-human lymphocyte serum, etc.

The binding of either porcine immunoglobulins or porcine monoclonal antibodies can be measured using the ELISA technique by binding the diluted animal (mouse, rat, etc.) monoclonal antibody to the semisolid phase of the ELISA system. The above described porcine or human antibody is then added in samples of different dilutions for incubation followed by an anti-human IgG or IgM conjugated to an enzyme. The semisolid phase is then separated from the samples and a color reagent (e.g., substrate) is added. The color of the sample is detected as an indication of the presence or absence of porcine monoclonal antibodies.

Preparation of Anti-Idiotype Antibodies

Both idiotypes and anti-idiotypes have been tested for manipulation of cellular as well as humoral immunity (Rajewski, K.S.., Takemori, T., Ann.

Rev. Immunol. 1:569, 1983). Recently, these observations have been used in the enhancement or induction of antibody responses to viral, bacterial, or parasitic antigens (Reagan, K.J., et al., J. Virol. 48:660, 1983; Kennedy, R.C., et al., Science 221:853, 1983; Kennedy, R.C., et al., J. Exp. Med. 159:655, 1984; Kennedy, R.C., et al., Virology 136:247, 1984; Ertl., H.C., et al., Proc. Natl. Acad. Sci. USA 81:2850, 1984; Ertl., H.C., et al., J. Exp. Med. 159:1778, 1984; Urbain, J., et al., In:Idiotypy in Biology and Medicine. G. Kohler, P.A., Cazenave, and J. Urbain (eds.) Academic Press, Inc., New York, p. 15, 1984; Francotte, M., et al., J. Exp Med. 160:1485, 1984; Sharpe, A.H., et al., J. Exp. Med. 160:1195, 1984; Uytadhaag, F.G., et al., J. Immunol. 134:1225, 1985; Gell, P.G., et al., J. Gen. Virol. 66:1801, 1985; Kennedy, R.C., et al., Science 232:220,1986; Urbain, J., et al., Ann. Immunol. 133D:179, 1982; McNamara, M.K., et al., Science 226:1325, 1984; Stein, K.E., et al., J. Exp. Med. 160:1001, 1984; Kaufmann, S.H., et al., J. Immunol. 134:4123, 1985; Sacks, D.L., et al., J. Exp. Med. 155:1108, 1982; Sacks, D.L., et al., J. Immunol. 131:1511, 1983; Sacks, D.L., et al., J. Immunol. 135:4155, 1985; and Grzych, J.M., et al., Nature 316:74, 1985). The systems described exploit the function of regulatory idiotypes (Francotte, M., et al., J. Exp. Med. 160:1485, 1984) or of related epitopes (Urbain, J., et al., In:Idiotypy in Biology and Medicine. G. Kohler, P.A. Cazenave and J. Urbain (eds.) Academic Press, Inc., New York, p. 15, 1984; Sharpe, A.H., et al., J. Exp. Med. 160:1195, 1984) in the normal immune response to pathogens.

A monoclonal anti-idiotype antibody can be a safe and effective prophylaxis for immunization and, therefore, can be used as a vaccine. The production of anti-idiotype antibodies by producing purified polyclonal anti-antibodies from porcine blood or by producing porcine/porcine, porcine/primate, or porcine/human hybridomas yielding anti-monoclonal antibodies provides anti-idiotype antibodies. Porcine/human anti-idiotype monoclonal antibodies can be used as a vaccine with a high degree of safety and minimal xenosensitization.

The anti-idiotype antibodies can be produced by initially immunizing an animal against a predetermined antigen, and recovering therefrom polyclonal or monoclonal antibodies to the antigen; subsequently, a second animal is immunized with the antibodies, and thus obtaining and recovering the desired anti-antibodies from the second animal. Desirably, the different breeds of pig described hereinabove are used for the first and second animals thus immunized, e.g., a minipig can first be immunized, followed by a mixed Yorkshire breed pig. Alternatively, it is possible to utilize two different animal breeds for the successive immunizations, e.g., breed of horses, cows, donkeys, sheep, goats, or monkeys, in addition to different pig breeds.

It is preferred to utilize a pig breed for the second immunization, i.e., to produce porcine anti-antibody, and to prepare porcine/porcine or porcine/human monoclonal antibodies therefrom, when producing anti-idiotype antibodies as vaccines for human therapy. The administration of horse or cow

immunoglobulins, for example, to humans is less desirable since they frequently give rise to immunogenic reactions by the host. The antibodies can raise antibodies reactive with the anti-idiotype antibodies, and thus prevent subsequent reaction with the antigen. Hosts other than pigs can be used for the second animal breed when producing anti-idiotype antibodies for veterinary vaccines.

Preferably, the production of anti-idiotype antibodies (Ab2) is carried out by immunizing a minipig with the antigen, e.g., purified CMV are recovered when a sufficiently high titer of anti CMV (Ab1) is obtained. The spleen is removed and the immunocytes are recovered. Fusing the immunocytes with either porcine or human myeloma cells, as described above. Cloning selected hybridomas using the limiting dilution technique and detecting anti CMV Ab1 monoclonal antibodies. Selected clones are then propagated for production of IgG.

The IgG monoclonal antibody is then used for immunizing a Yorkshire mixed bred pig after using a purification technique such as that described by Fons, et al., (Fons, G.C., et al., J. Immunol. 134:1225, 1985). Other purification methods, such as those described by Sacks, et al. (Sacks, D.L., et al., J. Immunology 135:4155, 1985) can, alternatively, be employed.

Thereafter, keyhole limpet hemocyanin (KLH) (Sigma Chem. St. Louis, MO) is coupled to the immunogen in the manner described in the literature (Mishell, B.B., et al., Selected Methods in Cellular Immunology, W.D. Freeman, San Francisco p. 303, 1980).

In order to effect immunization, the anti-idiotype monoclonal antibody can be precipitated with alum prior to vaccination. Other suitable techniques, which can serve as effective inducers of T and B immunity, have been described in the literature (Sanchez, Y., et al., Infect. Immun. 30:728, 1980).

An advantage in using the pig for the production of anti-idiotype antibodies is that the anti-idiotypes made using the porcine cell will be less likely to provoke the host's immune system to produce xenosensitization than a murine cell. In addition, unlike genetically engineered proteins intended for use as vaccines - which lack the ability to retain a microbial antigen's carbohydrate moiety - the anti-idiotype vaccine of the invention incorporates the carbohydrate moiety. In the majority of vaccines such emoiety plays an important role in rendering the host immune to the particular microorganism for which the anti-idiotype is made. Therefore, the anti-idiotypes have an advantage over recombinant vaccines.

The invention is further illustrated by the following examples. Unless otherwise indicated, all parts and percentages specified in the examples or referred to in the preceding description are given by weight, and all temperatures are given in degrees Celsius.

Example 1

Preparation of Porcine Monoclonal Antibodies

Cytomegalovirus (CMV) is isolated using sucrose gradient centrifugation. The virus is solubilized in

0.1-1.0% Triton X100 in phosphate buffered saline (PBS) at a pH of 7.2-7.4. The solubilized CMV is emulsified in Freund's complete adjuvant and 100-200 ug (volume 1 ml). CMV protein is injected subcutaneously on several sites on the neck of a mixed bred Yorkshire pig for immunization.

After 14 days the pig is immunized with 100-200 ug solubilized CMV protein in Freund's incomplete adjuvant. After another 14 days the pig is immunized with 100-200 ug of CMV protein in PBS. The titer of the porcine anti CMV immunoglobulins is determined employing a CMV-Ab ELISA test kit on a Behring ELISA Processor II.

The pig is then given a booster dose of 100-200 ug solubilized CMV proteins, administered one-half subcutaneously and one-half intraperitoneally. The pig is sacrificed and the spleen is recovered 1-5 days after the booster. The spleen is cut into appropriate pieces and is teased by forceps or teased gently through a sterile sieve into culture medium (RPMI 1640) to recover the spleenocytes. The spleenocytes are washed twice in RPMI 1640 and re-suspended in RPMI 1640 to a cell concentration of about 5 x 10^6 cells/ml.

Polyethylene glycol 1500 (BKH cat. no. 29575) (10 g) is autoclaved in a glass bottle. After cooling, but while the PEG is still liquid, 10 ml of RPMI 1640 is added and thoroughly mixed. The pH is maintained slightly alkaline.

A human myeloma cell line, GM4672B (IgG2 kappa secreting, HRPT deficient, selectable with 6-thioguanine), is used for fusion and the cell line is obtained when it is in logarithmic growth phase.

The myeloma cells are collected and mixed with the spleenocytes in a 2:1 ratio, $2x10^8$ myeloma cells:$1x10^8$ spleenocytes. The cells are gently pelleted in a tissue culture tube.

The fusion is performed under sterile conditions in a 37°C waterbath using a modification of the protocol by Oi and Herzenberg (Selected Methods in Cellular Immunology pp. 351-372, San Francisco, W.H. Freeman, 1980). A milliliter of RPMI 1640 (IMDM can also be used) containing 50% v/v polyethylene glycol (PEG 1500-Aldrich Chemicals) and 5% dimethylsulfoxide (DMSO-Baker Chemical Co.) is added dropwise over one minute to the tissue culture tube with constant stirring for two minutes. Subsequently, nine milliliters of warm (approximately 37°C) RPMI 1640 is added to the tissue culture tube over a time span of five minutes. The cell suspension is washed once using RPMI 1640 medium and centrifuged at 180 xg for ten minutes (23°C).

The cells are gently resuspended in 50 ml. RPMI 1640 containing 15% fetal calf serum (warm, 37°C) and 2% sterile filtrated piglet serum is added to the cell suspension. Aliquots (100 ul) of the cell suspension are dispensed into each well of five 96 well microtiter plates. The plates are incubated (5% CO_2, 95% atmospheric air, humidified) at 37°C. Every third day, the culture plates are fed with HT medium (no aminopterin) containing 15% fetal calf serum and 2% porcine serum. The cells are fed by replacing one-half of the supernatant in each well with fresh medium. The culture plates are fed on day four with HAT medium containing 15% fetal calf serum and 2% pig serum).

After about 8-14 days incubation, the hybrid colonies are screened for CMV antibody activity. Those showing desirable reactivities are transferred to new 24 well tissue culture plates (Falcon or Nunc), allowed to grow to confluence (3-5 days), and thereafter cloned.

The hybridoma colonies are cloned using a limiting dilution technique within two weeks of fusion, and a milliliter of well-mixed cell suspension is extracted from the well(s) containing the antibody of interest. Serial dilutions are made to obtain about 10 cells/ml in RPMI 1640, 10% fetal bovine serum (FBS), and a milliliter is seeded onto each well in 24 well plates, containing 10^5/ml of feeder cells. The culture is closely monitored for the emergence of clones (5-7 days) and tested for confluence. The supernates are tested for CMV antibodies.

The clone showing desirable antibody titer is then explanted into tissue culture bottles and maintained on RPMI 1640, 10% FBS. The supernate containing the antibody is harvested.

The porcine monoclonal antibody is purified on a Sepharose CNBR affinity column coupled to anti-human IgG (or antipig IgG). In this manner, a purified porcine monoclonal antibody (Ab1) is obtained in the eluate. The purified monoclonal antibody (MoAb1) can then be used in <u>in vitro</u> or <u>in vivo</u> diagnostic assays, e.g., in the passive immune treatment of CMV infections.

Example 2

Preparation of Porcine Monoclonal Antibodies Reactive with Tumor Necrosis Factor Alpha

A Yorkshire mixed bred pig was immunized with 20 mg per vaccination of recombinant Tumor Necrosis Factor Alpha (Cathectin), hereinafter TNF-alpha. The pig was immunized every fourteenth day with TNF-alpha. The initial vaccination was with "boiled" inactivated TNF-alpha mixed with Freund's Complete Adjuvant. Subsequently, "boiled" inactivated TNF-alpha was used for odd numbered vaccinations (e.g., 1, 3, 5) and non-activating TNF-alpha was used for even numbered vaccinations (e.g., 2, 4, 6). The polyclonal antibody titer was followed using a cytologic inhibition assay and when the pig showed a significant inhibition titer, a "booster" dose of TNF-alpha was administered intravenously. The intravenous booster contained 20 ug of TNF-alpha containing non-activated TNF-alpha in SDS without Freund's Adjuvant. Several months after the initial immunization, the spleen was removed from the pig, cut into small pieces, and placed into tissue culture tubes containing RPMI 1640 with L-Gluthamine and Pen-Strep.

GM4672B cells, Media, Reagents

A six week old piglet was bled and the serum was collected and inactivated at $56^{o}C$ for 30 minutes. The serum was then sterile filtrated as "supporter" for growth of the cells. GM4672B "conditioned" medium (RPMI 1640, containing 2 X 10 M 6-thioguanine, 1% L-Gluthamine, 15% Fetal Bovine Serum, and Pen.-Strep.), in which the GM4672B cells were grown, was collected as part of the feeder mechanism during

fusion. The GM 4672B cells in the tissue culture tubes were centrifuged at 800 rpm for five minutes. A 1% solution of HAT medium was added to the RPMI 1640 with the reagents used for growth during fusion period. A 50% solution of PEG 1000 was prepared and kept at 56°C prior to fusion.

Harvest of Spleenocytes (immunocytes)

The pieces of the porcine spleen were split up into small fractions and the spleenocytes were dispersed into the medium by gently teasing the cube (e.g., with forceps, forceps and scissors, using a sieve and a glass mortar). The cell suspension was examined and treated by suction and expulsion to prepare homogenous, smooth cell suspension. The cell suspension was then transferred into a tissue culture tube. The tube was filled with RPMI 1640 and the cell suspension was centrifuged at 800-1000 rpm for five to eight minutes. The cell pellet was re-suspended in a minimal amount of medium, depending upon the estimated amount of cells, and a cell count was performed. The cell count was done in a hema-cytometer using a microscope after cells were mixed with acetic acid and brilliant blue. The cell counts obtained varied from 1×10^8 to 1.9×10^8/ml. Microscopic examination of the cells revealed a low percentage of dead cells.

Fusion of Porcine Spleenocytes with Human Myeloma Cells

A ratio of two human myeloma (GM4672B) cells for each spleenocyte or one human myeloma (GM4672B) for each spleenocyte was used during fusion. Poly-ethylene glycol 1000 was slowly added dropwise to the cell mixture while gentle vortexing or gentle tapping

the cell mixture. RPMI 1640 (37°C) was added and the cells were gently treated with suction and expulsion with a pipette. The cells were then transferred to RPMI 1640 (37°C) with L-Gluthamine, 2% piglet serum, 15% FBS, 1% HAT, and Pen. Strep. yielding a cell concentration of approximately 1 x 10^6/ml.

Explanting of Fused Cells

The mixed cell suspension was explanted into six 96 well microtiter plates. A total of about 36,000 explants were subcloned to obtain hybrid colonies. The microtiter plates were then incubated.

Screening for Hybridoma Clones

The plates were fed after approximately eight to ten days with RPMI1640 containing the previously described sera. The wells were screened using invert microscopy and some of the wells showed growth of hybridoma cells after sixteen days with up to approximately 16 cell clones in wells containing live cells (hybridomas).

Screening of the Hybridomas for Clones Producing Antibodies Reactive with Tumor Necrosis Factor-alpha Clones

The hybridoma clones were screened using an Enzyme Linked Immunoassay (ELISA). Micotiter plates were coated with recombinant Tumor Necrosis Factor Alpha (Amgen) at 25 ng/ml and the plates were blocked with albumin. The polyvalent antiserum from the immunized pig previously tested for the presence of neutralizing TNF-alpha activity, was used as the control. The porcine serum contained 1000 neutralizing units of anti TNF-alpha per ml. The background

reading of the ELISA plates was 0.002. The polyvalent porcine anti TNF-alpha was used at a dilution of 1:25 to give an optical density (O.D.) reading of 0.10 which constitutes the control. Of 2640 clones screened, 21 clones were found to be anti-TNF-alpha positive. Fifteen of the clones produced antibodies which reacted with goat anti-porcine IgG conjugated to horse radish peroxidase. Six of the clones produced antibodies wihch reacted with anti-human IgG. One of the fifteen other clones showed definitive cross-reactivity with anti-human and anti-porcine conjugates. One of the six clones reacting only with anti human IgG conjugated to horse radish peroxidase and had an O.D. reading of 0.370 which is considered significantly high compared to the polyvalent pig serum control. The other clones had O.D. readings at between 0.060 to 0.090.

Cytotoxicity Neutralization Assay

The pig polyvalent antiserum was assayed for neutralizing antibodies to TNF-alpha. using a solid phase immunoassay. The polyvalent porcine anti TNF-alpha had a neutralizing titer of 1000 units/ml.

Non-Antibody Producing Hybridoma Cells

During the fusion of the porcine spleenocytes and human myeloma cells it was discovered that some of the porcine/human fused cells not capable of producing antibodies exhibited a good growth curve and seem to be very vital. This cell type was termed a non-antibody producing hybridoma and can be used as fusion partner in somatic cell hybridization methods in lieu of a human myeloma cell line.

Example 3

Preparation of A Porcine Monoclonal Antibody Reactive With A Murine Monoclonal Antibody

OKT3 (mouse monoclonal, Orthoclone IKT3, Ortho Pharmaceutical Corp., Raritan, N.J.), a potent T cell immunosuppressive agent, mixed with Freund's complete adjuvant, is administered (100ug-500ug) subcutaneously (s.c.) in split doses on the neck of a mixed Yorkshire bred pig. Forteen days thereafter the pig is revaccinated with OKT3 (100-500ug) s.c. (OKT mixed with Freund's incomplete adjuvant). After another 14 days the pig is given a third vaccination, and the titer of the antibody to murine immunoglobulins IgG2a (Bach, J.F., et al., Transplantation Proceeds 19:17,1987) is measured using the ELISA technique. Alternatively, the pig can be immunized with murine IgG2A non-OKT3 immunized. When a desirable antibody titer is obtained, the pig is sacrificed and hybridomas are produced employing the technique described in Example 1.

The antimouse IgG2a Mo Abl can be used, after purification as described in Example 1, for administration to patients injected with Orthoclone OKT3, in order to prevent the patients' immune system from producing antibodies against the Orthoclone OKT3.

Example 4

Preparation of Anti-Idiotype Antibodies

A minipig is immunized with CMV solubilized in Triton X100 (0.1-1.0 %) in Phosphate Buffered Saline (PBS) buffer. CMV, 100-200 ug/ml, in a total volume of 1 ml, is mixed with Freund's Complete Adjuvant.

The CMV is given in split doses, subcutaneously in the neck region and intraperitoneally.

The pig is re-vaccinated after fourteen days with 100-200 ug solubilized CMV in Freund's incomplete adjuvant, subcutaneously. The third immunization with solubilized CMV in PBS is given s.c. after another fourteen days.

The CMV antibody is measured using a CMV Ab ELISA test kit and the pig is sacrificed and bled. Serum from the pig is then partially purified with PEG precipitation (Carter, R.J. & Boyd, N.D., J Immunology Methods 26:213, 1979). The IgG is further purified using a Protein A Sepharose purification according to the method described by Sacks, et al., J. Immunology, 135:4155, 1985.

A mixed Yorkshire bred pig is immunized with the anti CMV (Ab1) according to the protocol described by Sacks and Sher (Sacks, D.L. & Sher, A.J., Immunology 131:1511, 1983). The IgG Ab2 is then biotinylated (biotin coupled to the antibody). The Ab1 is dotted on nitrocellulose paper at a volume of 1-3 ml, in 1:10, 1:100, 1:1000 dilutions in PBS Tween. Avidin peroxidase is reacted with the biotinylated (biotin and avidin peroxidase is reacted with the biotinylated (biotin and avidin peroxidase from Calbiochem) antibody for 30 minutes at a dilution of 1:5000. After washing, DAB substrate (12.5 mg/25 ml PBS) is added for color reaction.

When the pig is sufficiently immunized, it is sacrificed and the spleen is recovered. The immunocytes are isolated and fused, employing the protocol described in Example 1. The hybridomas showing the presence of Ab2 are then cloned by

limiting dilution. The Ab2-producing clone is then propagated.

The supernate is harvested and purified by Sepharose CNBr affinity chromatography, in which Ab1 to CMV is coupled. The Ab2 (anti-anti CMV) is eluted and the Ab2 can then be used for vaccination of humans, using Ab2 preparations described by Gaulton, et al., (Gaulton, G.N., et al., J. Immunology 127:2930, 1986).

The production of neutralizing antibodies, in this case "anti CMV production", requires a lengthy (60 days minimum) protocol (Gaulton, G.N., et al., J. Immunology 137:2930, 1986).

Example 5

Anti-idiotype Antibodies Containing an Internal Image Component

Human antibody to HbsAg serves as the idiotype or first antibody (Ab1). IgG is isolated from the serum using affinity chromatography on a Protein-A Sepharose column (Pharmacia). The IgG is conjugated to 50 ug keyhole limpet hemocyanin (KLH) (Calbiochem, La Jolla, CA) via 0.1% glutaraldehyde. (50 ug KLH to 50 ug IgG); see the method described by Kaminski, et al., (Kaminski, M.S., et al., J. Immunol. 136:1123, 1986).

IgG KLH (200-500 ug)is mixed with Freund's Complete Adjuvant and injected on several sites on the neck of a mixed bred Yorkshire pig. After fourteen days, the pig is given 200-500 ug IgG-KLH in Freund's incomplete adjuvant; after another 14 days, the pig is immunized with IgG-KLH in PBS at pH 7.4.

The HbSA9 Ab2 (anti-idiotype) was tested in an HbSA9 ELISA test. The pig is sacrificed when a significant titer is assayed. Hybridomas (porcine/human) are prepared as in Example 1.

The monoclonal anti-anti HbS antibody (MAb2) is obtained by cloning selected porcine/human hybridomas using a limiting dilution technique and recovering the monoclonal antibody from the supernate, as described by Tong, et al., (Tong, A.W., et al., Cancer Res. 44:4987, 1984). The MAb2 is purified on a Sepharose CNBr 4B gel to which is coupled a rabbit anti HbS Ab. The purified MAb2 is useful as a human vaccine.

Example 6

Anti-Idiotype Antibody Made from Murine Monoclonal Antibody

A murine monoclonal antibody to CMV (Mouse IgG) is purified using the method described by Perosa, et al., (Perosa, et al., J. Immunol. 138:2202, 1987; Russo, C., et al., J. Immunol. Methods 65:269, 1983; Ey, P.L., et al., Immunochemistry 15:429, 1978). After purification, the murine monoclonal antibody (murine MoAb1) is further purified as described by Perosa, et al. (Perosa, F., et al., J. Immunol. 138:2202, 1987), and is coupled to KLH polymerized with glutaraldehyde as described by Buttin, et al., (Buttin, G., et al., Curr. Top. Microbiol. Immunology 81:27, 1978).

200 ug of the thus treated murine MoAbl Anti-CMV is mixed with Freund's complete adjuvant, and utilized to immunize a Yorkshire mixed bred pig, in the neck regions. The pig is boostered 14 days after

the first vaccination with another 200 ug of the mouse MoAb1 mixed in Freund's incomplete adjuvant. After another 14 days, the pig is again boostered with the murine MoAb1 (200 ug) in PBS buffer.

The porcine MoAb2 (anti-idiotype antibody, viz., anti-anti CMV) is then bound as the semisolid phase on an ELISA microtiter place (96 wells) in different dilutions, from 1:10-1:10,000. Dilutions of the murine MoAb1 are then added to the plate for incubation (1 hour, R.T.). After removing the excess unbound supernate by suction and washing, a rabbit anti-human IgG-HRP conjugate (Calbiochem.) is added in different dilutions ranging from 1:500 to 1:10,000. The plate is incubated for another 60 minutes at R.T., and O.P.D. substrate is added (Electro Nucleonics).

The color reaction is read on a Behring ELISA Processor II at 492 nm. When a sufficiently high titer (i.e., between 0.400 and 2.200, or higher) is read, the pig is given a 200 ug booster dose of MoAb2, split one-half subcutaneously and one-half intravenously.

The spleen is recovered and the immunocytes (plasma cells) are isolated and fused with a human myeloma cell, as described in Example 1. The resulting hybridomas are cloned by limiting dilution, as described by Oi and Herzenberg (Oi, V.T. & Herzenberg, L.A. In: B.B. Mishell and S.M. Shiigi (eds.), Selected Methods in Cellular Immunology, pp. 351-372, San Francisco, W.H. Freeman Co., 1980).

The porcine MoAb2 (anti-idiotype, anti-anti CMV) is then purified from the supernate of the selected hybridoma clone, using affinity chromatography on

Sepharose CNBr 4B coupled with mouse MoAb1 against CMV. The eluate is the purified anti-idiotype porcine MoAb2, which may thereafter be used for vaccination of humans.

As described in Example 5, anti-idiotype antibodies (MoAb2) can be raised in pigs immunized with murine monoclonal antibodies. In the same manner, the pig can be immunized with other monoclonal antibodies. Thus, a porcine/human MoAb2 can be raised of any type of antigen/epitope which may be preferred for vaccination for any therapeutic or diagnostic use. Accordingly, murine monoclonal antibodies MoAb1 can be translated to porcine/human MoAb2, and murine monoclonal antibody MoAb2 can be translated to porcine/human MoAb1.

Alternatively, a murine monoclonal MoAb1 can be translated to a porcine/human monoclonal MoAb1 by immunizing another breed of animal, e.g., a rabbit (or a syngeneic mouse), with a murine monoclonal MoAb1. The rabbit is then capable of producing a rabbit antibody as an anti-idiotype (rabbit Ab2). The rabbit Ab2 anti-idiotype antibody can then be employed, towards which porcine antibodies (porcine Ab1) or porcine/human monoclonal antibodies (porcine/human MoAb1) can be raised. In this way, any porcine monoclonal antibody MoAb1 which cannot be readily used in human therapy can be translated to a murine antibody, porcineAb1, or to a porcine/human MoAb1.

The same translation mechanism can be used if a murine monoclonal antibody MoAb2 is to be translated to a porcine antibody, porcine Ab2, or to porcine/human MoAb2. The murine monoclonal MoAb2 is

used for immunization of another animal (a syngeneic mouse, a rabbit or any other animal) to raise a murine MoAb1 or a rabbit Ab1, which is then used for immunization of the pig to raise a porcine Ab2 or a porcine/human MoAb2.

It should be understood that other animal's monoclonal MoAb1 or MoAb2 can be translated to a porcine Ab2, and/or to a porcine/human MoAb1.

It will also be appreciated that synthetic peptides or recombinant peptides or proteins can be translated to a porcine Ab2 or to a porcine/human MoAb2 by immunizing a mouse, obtaining a monoclonal MoAb1, and raising antibodies in the porcine/human MoAb2. Instead of raising a murine monoclonal MoAb1 for immunization of a pig, a rabbit (or another breed of animal) can be immunized to raise an Ab1 antibody which again can be immunized in a pig to give a pig Ab2 or a pig/man MoAb2.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, with no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. These equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method for producing porcine antibodies reactive with a predetermined antigen, comprising:
   a) immunizing a pig with an antigen under conditions whereby porcine antibodies reactive with the antigen are produced;
   b) harvesting cells containing the porcine antibodies reactive with the antigen by collecting blood from the pig; and
   c) separating the porcine serum containing the antibodies from the other blood components.

2. A method according to Claim 1 wherein the antigen is a virus, a bacterium, a tumor antigen, a T3 lymphocyte, or a murine monoclonal antibody.

3. A method according to Claim 1 wherein the antigen is a cytomegalovirus or Tumor Necrosis Factor-alpha.

4. The method of Claim 1 wherein the pig is immunized with a murine monoclonal antibody to produce a porcine antibody in the pig reactive with the respective type of murine monoclonal antibody.

5. A passive immunotherapeutic method of treating a disease, comprising administering to a patient a therapeutically effective dosage of porcine antibodies.

6. A passive immunotherapeutic method of treating a disease comprising, administering to a patient a therapeutically effective dosage of a porcine anti-murine antibody.

7. A method for forming hybridomas capable of producing antibodies reactive with a pre-determined antigen, comprising:
   a) immunizing a pig with an antigen under conditions whereby porcine antibodies reactive with the antigen are produced;
   b) recovering antibody-producing cells from the pig's spleen;
   c) fusing the porcine antibody-producing cells with immortalizing cells, under conditions whereby fusion occurs;
   d) eliminating the unfused, residual porcine antibody-producing cells and immortalizing cells; and
   e) selecting the hybridomas capable of producing monoclonal antibodies reactive with the predetermined antigen.

8. A method according to Claim 7 wherein the immortalized cells are myeloma cells from a pig, human or other primate.

9. The method of Claim 7 wherein the pig is immunized with a murine monoclonal antibody to produce antibodies in the pig reactive with the respective type of murine monoclonal antibody.

10. The method of Claim 7 wherein the porcine antibody-producing cells are plasma cells and the immortalizing cells are human myeloma cells.

11. Hybridomas produced by the method of Claim 6.

12. A hybridoma formed by fusing a porcine antibody-producing cell with an immortalizing cell.

13. A method for producing monoclonal antibodies reactive with a predetermined antigen, which comprising:
   a) immunizing a pig with an antigen under conditions whereby porcine antibodies reactive with the antigen are produced;
   b) recovering porcine antibody-producing cells from the pig's spleen;
   c) fusing the porcine antibody-producing cells with immortalizing cells;
   d) eliminating unfused, residual porcine antibody-producing cells and immortalizing cells;
   e) cloning selected hybridomas capable of producing the desired monoclonal antibodies under conditions whereby monoclonal antibodies reactive with the antigen are produced; and
   f) recovering the monoclonal antibodies from the hybridoma supernate.

14. The method of Claim 13 wherein the pig is immunized with a murine monoclonal antibody to produce an antibody in the pig reactive with the respective type murine monoclonal antibody.

15. Monoclonal antibodies produced by the method of Claim 13.

16. A monoclonal antibody produced by a porcine antibody producing cell.

17. A passive immunotherapeutic method of treating a disease, comprising administering to a patient a therapeutically effective dosage of a porcine monoclonal antibody.

18. A method for producing anti-idiotype antibodies to a predetermined antigen, comprising:
    a) immunizing a first animal with an antigen under conditions whereby antibodies reactive with the antigen are produced;
    b) harvesting antibodies reactive with the antigen by collecting blood from the first animal;
    c) separating the antibodies from the blood;
    d) immunizing a second animal with the antibodies, under conditions whereby anti-antibodies are produced; and
    e) recovering the anti-antibodies from the blood of the second animal, wherein at least one of the first or second animals is a pig.

19. The method of Claim 18 wherein the first animal is a horse, cow, donkey, sheep, goat, monkey or a first breed of pig, and the second animal is a second breed of pig.

20. The method of Claim 18 wherein the first animal is a first pig breed, and the second animal is a second pig breed.

21. The method of Claim 18, wherein the anti-antibodies are monoclonal antibodies which are recovered in step (e) by:
   f) recovering the spleenocytes from the second animal containing the anti-antibodies;
   g) fusing the spleenocytes with immortalizing cells thereby producing hybridomas;
   h) eliminating the unfused, residual spleenocytes and immortalizing cells;
   i) cloning the hybridomas capable of producing anti-idiotype antibodies; and
   j) recovering the monoclonal antibodies from the hybridoma supernate.

22. A method for vaccinating a patient afflicted with a disease caused by a particular antigen comprising administering to the patient a therapeutically effective dosage of a vaccine containing porcine anti-idiotype antibodies.

23. In a method for the therapeutic treatment of a patient comprising, administering to the patient a murine monoclonal antibody against a predetermined antigen, the improvement which comprises additionally administering to said patient a porcine anti-murine antibody.

24. In a method for the therapeutic treatment of a patient the improvement comprising, administering a porcine antibody reactive with an antigen associated with the disease.

25. A chimeric monoclonal antibody comprising a porcine antigen binding region and a constant human region.

26. A method for treating an individual afflicted with a Tumor Necrosis Factor-alpha mediated disease comprising, administering to the individual a therapeutically effective dosage of a porcine monoclonal antibody reactive with Tumor Necrosis Factor-alpha such that the antibody neutralizes or minimizes the effects of the Tumor Necrosis Factor-alpha in the individual.

27. A method for treating an individual afflicted with a cytomegalovirus mediated disease comprising, administering to the individual a therapeutically effective dosage of a porcine monoclonal antibody reactive with cytomegalovirus such that the antibody neutralizes or minimizes the effects of the cytomegalovirus in the individual.

28. A non-antibody producing hybridoma cell useful as a fusion partner with an antibody-producing cell, comprising a non-antibody porcine producing cell fused to an immortalizing cell.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/03240

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: C 12 P 21/00, C 12 N 5/00, A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^5	C 12 P, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0200231 (STROSBERG) 5 November 1986 --	
A	Applied and Environmental Microbiology, volume 45, no. 1, January 1983, American Society for Microbiology, D. Thouvenot et al.: "Radioimmuno-assay for zearalenone and zearalanol in human serum: production, properties, and use of porcine antibodies", pages 16-23	

Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16th October 1989	22.11.89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V.[X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.[X] Claim numbers __*__, because they relate to subject matter not required to be searched by this Authority, namely:

* Claim numbers 5-6,17, 22-24, 26, 27
See PCT Rule 39.1.iv
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2.[ ] Claim numbers ______, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.[ ] Claim numbers______, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.[ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1.[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.[ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8903240

SA 30296

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 14/11/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0200231	05-11-86	JP-A- 62029972	07-02-87

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82